3. Tovey, G. H., Lockyer, J. W., Blades, A. N., Flarell, H. C. G., ibid., 1963, v8, 251.
4. Yokoyama, M., Fong, S., Fudenberg, H. H., Fed. Proc., 1963, v22, 381.
5. Polley, M. J., Adinolfi, M., Mollison, P. L., Vox Sang., 1963, v8, 385.
6. Yokoyama, M., Fudenberg, H. H., J. Immunol., 1964, v92, 413.
7. ———, ibid., 1964, v92, 966.
8. Haber, G., Rosenfield, R. E., Andresen, P. H., Festskrift, Munksgaard, Copenhagen, 1957, p45.
9. Landsteiner, K., Miller, C. P., J. Exp. Med., 1925, v42, 853.
10. Levy, H. B., Sober, H. A., Proc. Soc. Exp. Biol. and Med., 1960, v103, 250.
11. Friedenreich, V., With, S., Z. Immunforsch., 1933, v78, 152.
12. Wiener, A. S., J. Immunol., 1951, v66, 287.
13. Witebsky, E., Blood, 1948, v3, 66.

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Anti-Inflammatory Activity of Nonsteroidal Agents in a Rat Granuloma Assay. (30320)

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The cotton pellet anti-inflammatory bioassay, although lacking in precision as an ideal procedure, has served as a useful tool to find therapeutically useful anti-inflammatory steroids. This method has been applied to evaluation of nonsteroidal compounds as anti-inflammatory agents and the results reported here indicate significant anti-inflammatory activity for 6 nonsteroidal compounds.

Methods and materials. Twenty-three- to twenty-five-day-old albino rats of the Charles River strain were bilaterally adrenalectomized under ether anesthesia. At the time of surgery 2 cotton balls, each weighing 5 ± 1 mg, were inserted subcutaneously. From the time of surgery the operated animals were fed a commercial complete diet in the form of a Purina chow and 0.9% saline was substituted for drinking water. The test compounds were dispersed in a vehicle consisting of sodium chloride (0.9%), polysorbate 80 (0.4%), carboxymethylcellulose (0.5%) and benzyl alcohol (0.9%) so that the total dose was contained in 0.2 ml. One day and 2 days after surgery the test animals received subcutaneous injections of 0.1 ml of the suspensions. Rats of the control groups received a total of 0.2 ml of the vehicle only. Twenty-four hours after the last injection, the rats were sacrificed with ether, the granuloma surrounding the cotton pellets was removed, the body weights were determined. The dissected granulomas were dried for 24 hours at 100°C and the dry granuloma weight was obtained by subtracting the weight of the cotton pellet from the gross weight. In most trials each of the 2 granulomas in a given animal were considered to be individual observations. In some experiments the sum of the 2 granuloma weights was treated as a single observation.

In most of the experiments the test material was administered by subcutaneous injection. In one trial the test compound and standard were incorporated into the diet and the dose expressed as mg of compound per kilogram of food. In these studies the experimental diet was fed for 48 hours.

Results and conclusions. Tables I through VIII summarize the anti-inflammatory activity of 6 compounds studied by the subcu-

TABLE I. Anti-Inflammatory Activity of Subcutaneously Administered Chlorpheniramine Maleate (CM).

Material administered	Total dose, mg	No. of observations	Mean body wt, g	Mean dry tissue wt, mg ± S.E.
O	0	20	57	16.5 ± 1.1
Cortisol acetate	.4	24	57	14.9 ± 1.1
	.8	20	57	11.9 ± 1.2
	1.6	21	55	10.8 ± .8
CM	1	21	53	15.7 ± 1.5
	5	20	52	11.4 ± 1.4

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taneous route, and one compound, aminopyrine, whose potency was studied both by subcutaneous injection and when admixed with food. Chloropheniramine maleate was assayed at total doses of 1 and 5 mg with the higher dosage showing a statistically significant decrease in the weight of the granuloma tissue (Table I). A total dose of 20 mg of the compound, not indicated in the table, was toxic to the adrenalectomized rats.

At the 30 mg total dose antipyrine exhibited a significant inhibition in granuloma size of the order of 50% without producing a significant change in body weight. The compound was ineffective at the 0.5 and 5.0 mg dose levels.

Subcutaneously injected aminopyrine produced only a suggestive inhibitory response in 2 different experiments and in a total of 4 concentrations (Table III), but when incorporated into the diet, even the smaller dose of 150 mg per kilo was inhibitory (Table IV).

TABLE II. Anti-Inflammatory Activity of Subcutaneously Administered Antipyrine (AT).

Material administered	Total dose, mg	No. of observations	Mean body wt, g	Mean dry tissue wt, mg ± S.E.
O	0	19	63	17.7 ± .8
Cortisol acetate	.2	20	65	25.0 ± 1.7
	.4	20	60	13.6 ± 1.3
	.8	20	62	15.2 ± 1.0
AT	.5	18	62	23.1 ± 2.0
	5	18	66	19.4 ± 2.0
O	0	9	57	39.2* ± 2.0
Cortisol acetate	.4	10	57	19.6 ± 2.1
	.8	10	59	14.7 ± 1.7
	1.6	10	50	14.2 ± 1.4
AT	30	10	57	18.8 ± 2.0

* This represents the sum of the weights of 2 granulomas in a single rat.

TABLE III. Anti-Inflammatory Activity of Subcutaneously Administered Aminopyrine (A).

Material administered	Total dose, mg	No. of observations	Mean body wt, g	Mean dry tissue wt, mg ± S.E.
O	0	34	57	17.1 ± .9
Cortisol acetate	.2	32	59	17.6 ± 1.1
	.4	32	55	16.7 ± 1.4
	.8	20	57	13.7 ± 1.1
A	.5	20	55	14.9 ± .9
	5.0	18	63	14.4 ± .6
O	0	18	59	18.0 ± .9
Cortisol acetate	.2	20	59	16.0 ± 1.3
	.4	20	63	18.2 ± 1.6
	.8	18	57	12.6 ± 1.0
A	15	16	60	19.7 ± 1.9
	30	16	61	14.2 ± 1.0

TABLE IV. Anti-Inflammatory Activity of Aminopyrine (A) Incorporated into the Food.

Compound	Dose, mg/kilo	No. of observations	Mean dry tissue wt, mg ± S.E.
O	0	18	27.9 ± 2.8
Cortisol acetate	25	20	20.7 ± 1.2
	50	18	15.9 ± 1.8
	100	18	11.8 ± .7
A	150	18	13.3 ± .6
	1250	18	14.1 ± 1.0

TABLE V. Anti-Inflammatory Activity of Subcutaneously Administered Cinchophen (C).

Material administered	Total dose, mg	No. of observations	Mean body wt, g	Mean dry tissue wt, mg ± S.E.
O	0	34	57	17.1 ± .9
Cortisol acetate	.2	32	59	17.6 ± 1.1
	.4	32	55	16.7 ± 1.4
	.8	20	57	13.7 ± 1.1
C	.5	20	61	18.7 ± .9
	5.0	18	60	16.8 ± 1.2
O	0	10	71	46.1* ± 2.3
Cortisol acetate	.4	9	64	28.5 ± 2.2
	.8	9	59	24.7 ± 1.5
	1.6	9	58	22.7 ± 2.3
C	30	9	58	31.8 ± 4.3

* This represents the sum of the weights of 2 granulomas in a single rat.

TABLE VI. Anti-Inflammatory Activity of Subcutaneously Administered Phenylbutazone (P).

Material administered	Total dose, mg	No. of observations	Mean body wt, g	Mean dry tissue wt, mg ± S.E.
O	0	12	54	19.3 ± 1.2
Cortisol acetate	.05	13	53	17.3 ± 1.1
	.4	13	49	13.4 ± 1.6
	1.6	13	48	9.2 ± 1.4
P	.4	11	56	18.9 ± 1.4
	5.0	13	50	14.0 ± .9
O	0	17	64	30.2 ± 2.7
Cortisol acetate	.2	18	63	25.8 ± 1.7
	.4	16	66	14.5 ± 1.8
P	7.5	16	60	25.3 ± 2.3
	15.0	17	56	19.4 ± 1.3

TABLE VII. Anti-Inflammatory Activity of Subcutaneously Administered Dipyrone (D).

Material administered	Total dose, mg	No. of observations	Mean body wt, g	Mean dry tissue wt, mg ± S.E.
O	0	16	64	24.2 ± 1.8
Cortisol acetate	.4	19	61	16.7 ± 2.2
	.8	20	59	15.3 ± 1.1
D	5	20	60	15.6 ± .7
	10	16	55	13.3 ± .9
	20	15	62	20.1 ± 2.1
O	0	19	63	17.7 ± .8
Cortisol acetate	.2	20	65	25.0 ± 1.7
	.4	20	60	13.6 ± 1.3
	.8	20	62	15.2 ± 1.0
D	.5	18	66	20.3 ± 1.7
	5	18	60	13.9 ± .9
O	0	18	62	26.2 ± 2.5
Cortisol acetate	.2	18	61	23.7 ± 2.0
	.4	18	60	18.7 ± 2.0
	.8	18	60	14.8 ± .6
D	5	18	58	21.1 ± 1.4
O	0	17	64	30.2 ± 2.7
Cortisol acetate	.2	18	63	25.8 ± 1.7
	.4	16	66	14.5 ± 1.8
D	7.5	18	61	18.6 ± 1.8
	15.0	18	63	16.2 ± 1.0

TABLE VIII. Granuloma Tissue Response to Cortisol Acetate.

Total dose, mg	No. of observations	Mean dry granuloma wt, mg
0	156	21.0
.2	126	21.3
.4	218	16.4
.8	134	14.0
1.6	72	11.5

Cinchophen produced a significant inhibition in the granuloma tissue growth at the 30 mg dose level (Table V), but at the 0.5 and 5.0 mg dose levels, no change occurred.

Phenylbutazone caused statistically significant granuloma tissue inhibition in one experiment at the 5 mg total dose level when the dry tissue weight was decreased from 19.3 ± 1.2 (S.E.) to 14.0 ± 0.9 (S.E.) and in a second experiment at the 15 mg dose level when the tissue weight was decreased from 30.2 ± 2.7 (S.E.) to 19.4 ± 1.3 (S.E.).

Eight groups of animals received dipyrone. At the 0.5 mg dose level no effect could be observed. The 5 mg dose level experiments did result in significant inhibitions. At the 7.5, 10 and 15 mg total dose levels statistically significant inhibitions were recorded (Table VII) but one trial at the dose of 20 mg was indefinite.

In each trial standard reference cortisol acetate was studied simultaneously with the test nonsteroid compound. A composite of cortisol acetate responses involving 706 observations is illustrated in Table VIII. This response of the reference standard was used to estimate the relative potencies of the nonsteroid compounds. This was admittedly most difficult, since no true dose response could be demonstrated for the latter compounds. The high variation is readily apparent from the wide ranges reported in Table IX.

Discussion. Phenylbutazone has been studied by the cotton ball granuloma assay by other investigators with results similar to those reported here. Winter *et al*(5) have reported phenylbutazone to be 0.05 to 0.1 as active as cortisol by the oral route. Winder *et al*(3,4) reported that phenylbutazone possesses anti-inflammatory activity in the cotton ball assay but made no attempt to express a relative potency of the nonsteroid.

In the present report we assign a relative potency of phenylbutazone to cortisol acetate by injection as 2-16%. The value for oral administration for phenylbutazone to cortisol was 5 to 10%. These values are of the same order as the relative potencies determined for humans, since the human dose of phenylbutazone in rheumatoid arthritis is about 400 mg per day, while that of cortisol is about 40 mg.

The cotton ball granuloma test seems to have some value for assay of nonsteroid anti-inflammatory agents. In addition to the au-

TABLE IX. Relative Potency of Various Subcutaneously Administered Nonsteroidal Anti-Inflammatory Compounds.

Compound	Range of total doses studied, mg	Graphically estimated relative potency range (cortisol acetate = 100)
Chloropheniramine maleate	1; 5	28–55
Dipyrone	5; 7.5; 15	3– 8
Phenylbutazone	5; 15	2–16
Aminopyrine	5; 30	2–14
Cinchophen	5; 30	2– 8
Antipyrine	5; 30	1– 5

thors already quoted, Steelman *et al*(2) report a modification of the original Meier method(1) which could detect phenylbutazone at doses of 200-2000 mg/kg of diet without influencing body, thymus, or adrenal weight. Winder *et al*(4) have described Cl-440; flufenamic acid (N-(*a*,*a*,*a*-trifluoro-m-tolyl) anthranilic acid) as being 3.6 (1.2-40) (95%) times as active as phenylbutazone in a subcutaneous cotton pellet test in rats.

Winder *et al*(3) used a modified Meier cotton pellet antigranulation method in intact and adrenalectomized rats for assay of Cl-473 (N-(2,3-xylyl) anthranilic acid, mefenamic acid) in comparison to phenylbutazone. The anti-inflammatory effects were considered to be independent of the adrenal glands. The compound by the oral route was judged to be 0.6 (0.1-2.1) (95%) as potent as phenylbutazone. The difference between the slopes for cortisol and phenylbutazone was so large that relative potencies could not be determined.

Winter *et al*(5) have reported a new compound, indomethacin [1-(p-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid] which is an orally active inhibitor of cotton pellet granuloma formation in both the intact and adrenalectomized rat administered either systematically or locally. The compound has a relative potency 85 times phenylbutazone and about 4 times cortisol.

To this group we now add chloropheniramine maleate, dipyrone, aminopyrine, cinchophen and antipyrine as anti-inflammatory agents as judged by the cotton ball granuloma assay method.

Summary. An anti-inflammatory granuloma assay in the adrenalectomized rat has been employed to evaluate the relative potency of various nonsteroidal compounds. Statistically significant responses have been observed, but dose-related responses were not obtained. Graphic estimations of the range of relative potencies using cortisol acetate as the standard with an assigned potency of 100 follows: chloropheniramine maleate, 28-55; dipyrone, 3-8; phenylbutazone, 2-16; aminopyrine, 2-14; cinchophen, 2-18; and antipyrine, 1-5.

1. Meier, R., Schuler, W., Desaulles, P., Experientia, 1950, v6, 469.

2. Steelman, S. L., Morgan, E. R., Silber, R. H., Steroids, 1963, v1, 163.

3. Winder, C. V., Wax, J., Scotti, L., Scherrer, R. A., Jones, E. M., Short, F W., J. Pharmacol. Exp. Therap., 1962, v138, 405.

4. Winder, C. V., Wax, J., Serrano, B., Jones, E. M., McPhee, M. L., Arthritis Rheumat., 1963, v6, 36.

5. Winter, C. A., Risley, E. A., Nuss, G. W., J. Pharmacol. Exp. Therap., 1963, v141, 369.

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Fatty Acid Composition of Sphingomyelin and Lecithin in Normal Human Serum.* (30321)

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The three major phospholipids in human blood serum are lecithin, lysolecithin and sphingomyelin. Of these, lecithin makes up approximately 67% of the serum phospholipids, while sphingomyelin and lysolecithin make up 21% and 8% respectively(1,2). Studies of the fatty acid composition of serum lecithin(3,4) indicate that it is comprised primarily of 16:0,† 18:0, 18:1 and 18:2 fatty acids. Human serum sphingomyelin, on the

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† The chain length of the fatty acid is given by the numeral before the colon and the number of double bonds by the numeral after it.